Pergamon

Tetrahedron Letters 41 (2000) 5881–5885

TETRAHEDRON LETTERS

Synthesis and antitumor activities of phosmidosine A and its *N*-acetylated derivative

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Received 23 March 2000; revised 25 March 2000; accepted 2 June 2000

Abstract

A new antitumor active phosmidosine A, which was isolated from *Streptomyces* sp., was successfully synthesized by a series of reactions involving construction of the *N*-acyl phosphoramidate linkage which was achieved by the reaction of the 5′-*O*-phosphoramidite derivative with a prolinamide derivative in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole. The growth inhibitory effect of phosmidosine A and its *N*-acetyl analog on the various human cell lines was examined. These results showed that both compounds have a significant growth inhibitory activity and that the 6-amino group is not required for the growth inhibitory activity of phosmidosine A.

Keywords: phosmidosine A; 8-oxoadenosine; L-proline; *N*-acyl phosphoramidate linkage; antitumor activity.

Phosmidosine (**1**) was found as a new type of antifugal antibiotic isolated from a culture filtrate of *Streptomyces durhameusis*[1] by Uramoto and Isono et al. Later, it was found by mass spectrometry and NMR spectroscopy that phosmidosine was a novel nucleotide-type antibiotic, having an *N*-acyl phosphoramidate linkage which connects a nucleoside analog, 8-oxoadenosine, with an L-proline residue.[2] Recently, Osada et al. have reported that phosmidosine suppresses S-phase entry and arrests cell cycle progression at the G_1 phase in human lung fibroblast WI-38 cells.[3] Moreover, phosmidosine A (**2**) and B were also isolated as compounds having the morphological reversion activity from the fermentation broth of *Streptomyces* sp. strain RK-16, which is a producer strain of phosmidosine.[4] It was shown that phosmidosine A (**2**) displays inhibitory activity against the cell cycle progression and the morphological reversion activity on *src*^{ts}-NRK cells in a manner similar to that described in the case of phosmidosine (**1**).[4]

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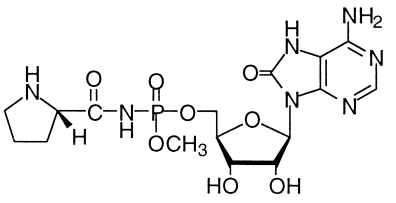
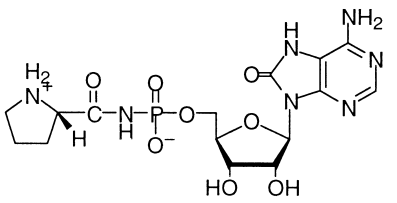
PII: S0040-4039(00)00926-6



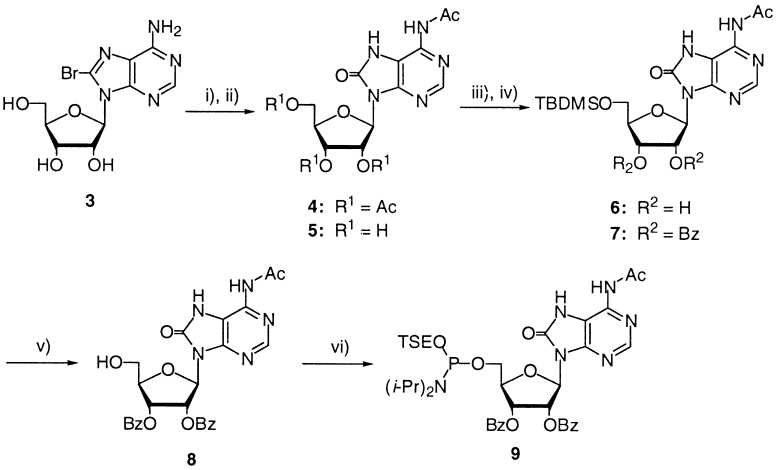
Phosmidosine (**1**)



Phosmidosine A (**2**)

It was reported that the *N*-acyl phosphoramidate linkage of phosmidosine is extremely unstable under weakly basic conditions,[1] therefore the base–labile protecting groups were not used for the synthesis of phosmidosine. However, the demethylated *N*-acyl phosphoramidate linkage has proven to be stable under both basic and acid conditions.[5a] In this paper, we report the synthesis and p53 phenotype-independent cytotoxicity of phosmidosine A, i.e. a demethylated derivative of phosmidosine. The key step for the synthesis of this natural product is the construction of the *N*-acyl phosphoramidate linkage. We have recently reported an effective method for the synthesis of a new type of aminoacyl-adenylate analog, having an *N*-acyl phosphoramidate linkage.[5] In this approach, the *N*-acyl phosphoramidate linkage was formed by condensation of adenosine 5′-phosphoramidite derivatives with amino acid amide derivatives in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole as an activating reagent of the phosphoramidite derivatives.

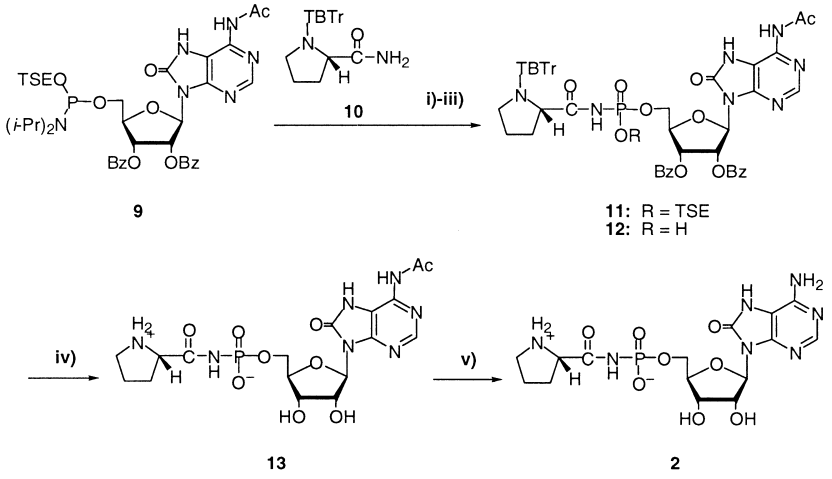
To synthesize phosmidosine A, we prepared the 5′-*O*-phosphoramidaite derivative **9** of 8-oxoadenosine as shown in Scheme 1. 8-Bromoadenosine (**3**) was hydrolyzed and the resulting 8-oxoadenosine derivative was acetylated by treatment with acetic acid and acetic anhydride in the presence of sodium acetate at 120°C.[6] The peracetylated derivative **4** was treated in situ with aqueous ammonia to give the *N*-acetylated 8-oxoadenosine derivative **5** in 83% yield, from **3**. The selective silylation of **5** with TBDMSCl in pyridine gave the 5′-*O*-TBDMS derivative **6** in 94% yield. Treatment of **6** with benzoic anhydride in the presence of DMAP gave the 2′,3′-di-*O*-benzoyl



Scheme 1. (i) AcONa, Ac_2O, AcOH, 120°C, 3 h; (ii) conc. NH_3–pyridine (9:1, v/v), rt, 6 h; (iii) TBDMSCl (1.2 equiv.), pyridine, rt, 12 h; (iv) Bz_2O (2.4 equiv.), DMAP (0.1 equiv.), pyridine, rt, 4 h; (v) TBAF·H_2O (1.3 equiv.), AcOH (1.3 equiv.), THF, rt, 12 h; (vi) TSEOP(N(*i*-Pr)$_2$)$_2$ (1.5 equiv.), diisopropylammonium tetrazolide (0.5 equiv.), CH_2Cl_2, rt, 1.5 h

derivative **7** in 88% yield, which was further converted by treatment with TBAF to the 5′-hydroxyl derivative **8** in 93% yield. It is known that phosmidosine of the *O,O*-dialkyl phosphoramidate type is extremely unstable even under weak basic conditions.[1] Therefore, the 2-(trimethylsilyl)ethyl (TSE) group[7] was used as a phosphate-protecting group that can be removed by treatment with TBAF under neutral conditions. The 5′-OH derivative **8** was allowed to react with 2-(trimethylsilyl)ethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite in the presence of diisopropylammonium tetrazolide to give the 5′-*O*-phosphoramidite derivative **9** in 81% yield.

As the protecting group of the amino group of prolinamide, the base-labile 4,4′,4″tris-(benzoyloxy)trityl (TBTr) group[8,9] was employed. We previously found that the solubility of amino acid amide derivatives is important for the smooth P–N bond formation between phosphoramidite derivatives and amino acid amide derivatives.[5] Moreover, this base-labile protecting group can be removed together with other acyl protecting groups at the same time. Thus, *N*-TBTr prolinamide (**10**) [5] was allowed to react with the 5′-*O*-phosphoramidite derivative **9** in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole. This reaction proceeded to give the condensation product, which was treated in situ with *t*-BuOOH to give the phosphoramidate derivative **11**. Further subsequent treatment of **11** with TBAF·H_2O and AcOH afforded the desired *N*-acyl phosphoramidate derivative **12** in 43% yield. It was found that the *N*-acetyl group remained after treatment of **12** with aqueous ammonia, and the *N*-acetylated derivative **13** was obtained in 72% yield. Additional treatment of **13** with 1 M NaOH at 70°C for 30 min gave the desired product, phosmidosine A (**2**), which could be isolated in 53% yield by C-18 reversed-phase chromatography (Scheme 2). The structure of **2** was confirmed by NMR spectroscopy and MALDI-TOF mass spectroscopy.[10]



Scheme 2. (i) **10** (0.67 equiv.), 5-(3,5-dinitrophenyl)-1*H*-tetrazole (2 equiv.), CH_3CN, rt, 10 min; (ii) *t*-BuOOH (3.3 equiv.), rt, 5 min; (iii) TBAF·H_2O (3 equiv.), AcOH (3 equiv.), THF, rt, 18 h; (iv) conc. NH_3–dioxane (1:1, v/v), rt, 8 h; (v) 1 M NaOH, 70°C, 30 min

The growth inhibitory activities of phosmidosine A (**2**) and the *N*-acetylated derivative (**13**) against various human cancer cells were tested in the MTT assay.[11] These results are summarized in Table 1, which also shows the data of the inhibitory activity of CDDP as a positive control.

Table 1
The growth inhibitory activities of phosmidosine A (**2**) and **13** against various human cancer cells[a]

cell line	origin	P53 phenotype				IC_{50} (μM) **2**	IC_{50} (μM) **13**	IC_{50} (μM) CDDP
		status	codon	nt change	aa change			
KB	Larynx	WT[b]	No	No	No	190.0	180.0	0.90
MKN-28	Stomach	mutant	251	ATC>CTC	Ile>Leu	130.0	128.0	3.68
MKN-45	Stomach	WT[b]	No	No	No	19.0	24.5	0.68
KATO III	Stomach	complete deletion	Loss	Loss	Loss	28.0	29.0	2.50
NUGC-3	Stomach	mutant	251	TAT>TGT	Tyr>Cys	17.0	17.0	1.10
NUGC-4	Stomach	WT[b]	No	No	No	8.8	15.0	0.65
SW-48	Colon	WT[b]	No	No	No	65.0	–[c]	0.86
LS174T	Colon	WT[b]	No	No	No	14.8	–[c]	0.64
SW-480	Colon	mutant	273 309	CGT>CAT CCC>TCC	Arg>His Pro>Ser	25.0	–[c]	2.00
HT-29	Colon	mutant	273	CGT>CAT	Arg>His	24.0	–[c]	6.00
SBC-3	Lung	WT[b]	No	No	No	98.0	–[c]	0.68
PC-9	Lung	mutant	248	CGG>CAG	Arg>Gln	10.8	–[c]	2.50

[a]The tumor cell growth inhibitory activity assay *in vitro* was done by the method described by Carmichael *et al.*[11] Each tumor cell line ($2x10^3$ cells/well) was incubated in the presence or absence of compounds for 72 h. The MTT reagent was added to each well and the plate was incubated for an additional 4 h. The resulting MTT-formazan was dissolved in DMSO and the OD value at 540 nm was measured. Percent inhibition was calculated as follows: % inhibition = [1 - OD (540 nm) of sample wells/OD of control wells] x 100. IC_{50} (μM) was given as the concentration at 50% inhibition of cell growth.
[b]wild type
[c]not tested

Phosmidosine A (**2**) showed significant growth inhibitory activities against the various human cancer cell lines in the range of 8.8–190 μM. Furthermore, the difference in antitumor activity between phosmidosine A and its *N*-acetylated derivative **13** was little observed in this assay. These results would be helpful for the design of phosmidosine analogs as antitumor agents. The antitumor activity of phosmidosine A is not influenced by the status of p53 phenotype in tumor cells, while CDDP showed significant selective inhibitory activity against the p53 wild type cell lines. Further studies are required to elucidate the mechanism of the p53-independent growth inhibitory action of phosmidosine analogs.

The synthesis of phosmidosine is now under investigation, and the structure–activity relationship between the phosmidosine and the antitumor activity is also being studied.

Acknowledgements

This work was supported by a Grant from 'Research for the Future' Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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10. Compound **2**: ^{1}H NMR (D_2O) 1.89–1.98 (3H, m, 4″-Ha, Hb, 3″-Ha), 2.39 (1H, m, 3″-Hb), 3.36 (2H, m, 5″-Ha, Hb), 4.07–4.18 (3H, m, 4′-H, 5′-Ha, Hb), 4.36 (1H, m, α-H), 4.53 (1H, dd, J = 5.3 Hz, J = 4.6 Hz, 2′-H), 5.11 (1H, dd, J = 5.3 Hz, J = 5.3 Hz, 3′-H), 5.85 (1H, d, J = 4.9 Hz, 1′-H), 8.09 (1H, s, 2-H); ^{13}C NMR (D_2O) δ 26.20 (4″-C), 32.09 (3″-C), 49.00 (5″-C), 63.02 (d, J = 13.4 Hz, α-C), 67.98 (d, J = 4.9 Hz, 5′-C), 72.38, 72.92 (2′-C, 3′-C), 84.84 (d, J = 8.5 Hz, 4′-C), 88.62 (1′-C), 122.07 (5-C), 149.38 (6-C), 150.15 (4-C), 153.94 (2-C), 155.51 (8-C), 173.46 (C=O); ^{31}P NMR (D_2O) δ –5.13 (s); MALDI-TOF mass Calcd for m/z $C_{15}H_{23}N_7O_8P$ $(M+H)^+$ 460.13. Observed for m/z 460.15.
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